Effects of Bisphenol A and Methoxychlor on *Xenopus Laevis* Embryos

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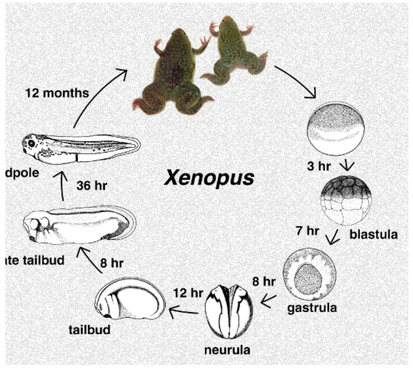
Background

Model Organism: Xenopus Laevis

Due to their aquatic reproduction, amphibians make an apt model to study the effects of aqueous environmental contamination on embryogenesis. Primordial germ cells (PGCs) are stem-like cells that will eventually differentiate into oocytes and sperm cells in the mature adult organism (Strome and Updike, 2015).

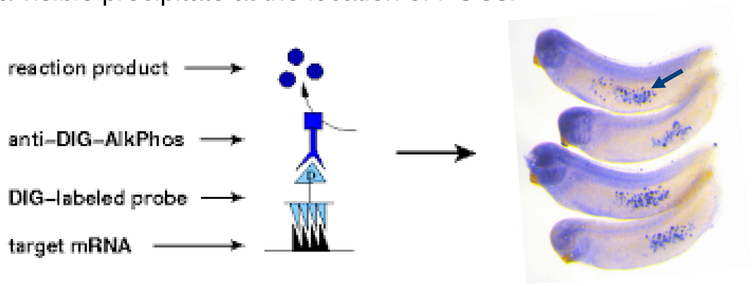


Bisphenol A is a synthetic monomer used for the production of polycarbonate plastics and epoxy resins and is found in numerous consumer products. Over six billion pounds of BPA is produced per year, resulting in the release of BPA into the environment (Seachrist et al., 2016; Howdeshell et al., 1999). Previous work has demonstrated the link between *Xenopus* embryonic exposure to BPA and alterations to the reproductive system and somatic tissue development (Levy et al., 2004; Pickford et al., 2003;Iwamuro et al., 2003; Adamakis et al., 2016; George et al., 2008; Sone et al., 2004). Methoxychlor is a pesticide that has shown to induce adverse effects such as poorly developed dorsal fins and depletion of melanocytes in developing *X. laevis* (Bevan et al., 2002)

Research Question: How does Bisphenol A and Methoxychlor effect the primordial germ cells in Xenopus Laevis embryos

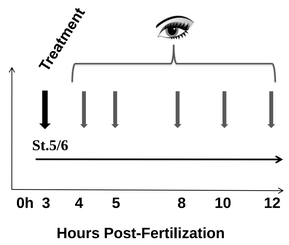
Approach

Primoridal germ cells are visualized via PGC marker *xpat*. Embryos are incubated with an mRNA probe complementary to *xpa*t and tagged with the protein Digoxigenin (DIG). An an antibody for DIG is conjugated with alkaline phosphatase and allows for the formation of a visible precipitate at the location of PGCs.



Bisphenol A

(A)

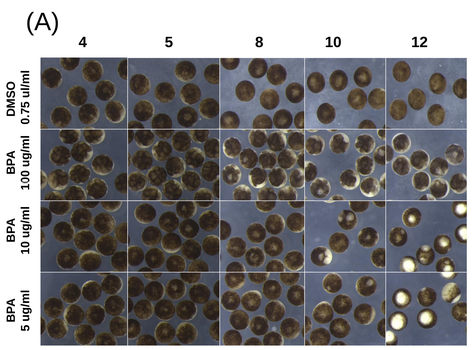


(B)

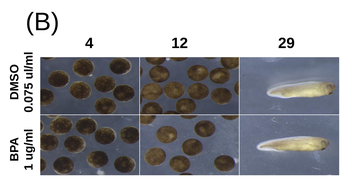
	Total	3 hpf	4 hpf	5 hpf	6 hpf	7 hpf	8 hpf	10 hpf	12 hpf
BPA 100 µg/ml	25	0	0	0	0	0	8	0	92
BPA 10 µg/ml	21	0	0	0	0	0	0	28.6	71.4
BPA 5 µg/ml	26	0	0	0	0	0	0	23.1	76.9
BPA 1 µg/ml	26	0	0	0	0	0	0	0	0
DMSO 0.75 ul/ml	23	0	0	0	0	0	0	0	0

(A) Experimental Schematic: Monitoring embryo response to various BPA concentrations (B) Percentage of dead *Xenopus laevis* embryos after exposure to 100 µg/ml, 10µg/ml, 5 µg/ml, and 1µg/ml BPA starting at 3 hours post fertilization.

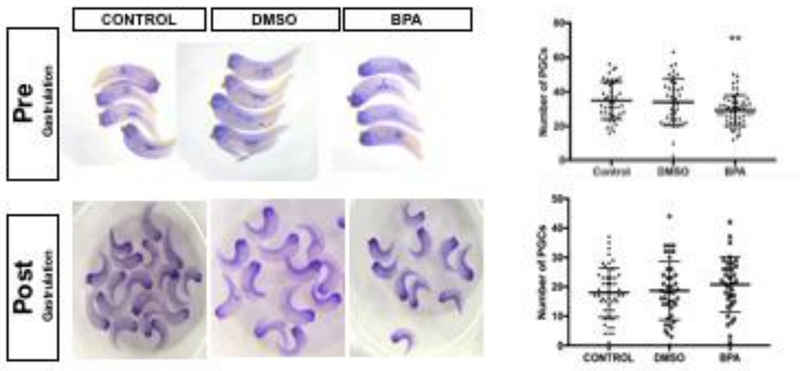
(A)



(B)



(A) Representative images of *Xenopus laevis* embryos at 4, 5, 8, 10, 12 hours post fertilization that were exposed to a DMSO or BPA at 100µg/ml, 10µg/ml, or 5µg/ml. (B) Representative images of Xenopus laevis embryos exposed to 1µg/ml at 4, 12, and 29 hours post fertilization. (C). Embryos were exposed to DMSO control or BPA at concentrations of 1µg/ml, 2.5µg/ml, 5µg/ml, and 10 µg/ml.

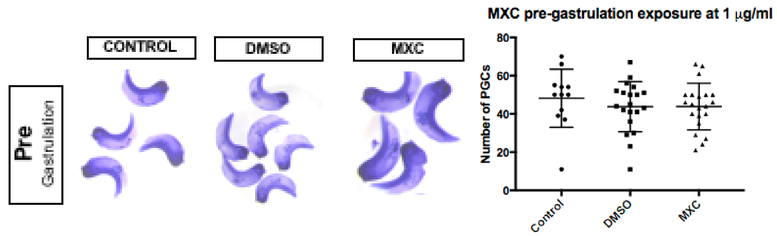


Representative images of embryos exposed to 1µg/ml BPA before and after gastrulation. PGCs labeled via in situ hybridization for marker Xpat. Number of total PGCs counted

Methoxychlor

	Total	3 hpf	4 hpf	5 hpf	6 hpf	7 hpf	8 hpf	10 hpf	12 hpf
MXC 100 µg/ml	23	0	0	0	0	0	0	0	0
MXC 50 µg/ml	25	0	0	0	0	0	0	0	0
MXC 10 µg/ml	22	0	0	0	4.5	0	0	-	0

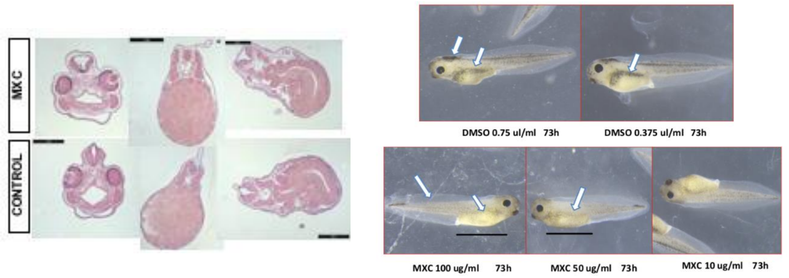
MXC toxicity: Percentage of dead *Xenopus laevis* embryos after exposure to 100 µg/ml, 50µg/ml, and 10 µg/ml MXC starting at 3 hours post fertilization.



Representative images of embryos exposed to 1µg/ml MXC before and after gastrulation. PGCs labeled via in situ hybridization for marker *xpat*. Number of total PGCs counted

Conclusions and Future Work

BPA at concentrations higher than 1 ug/ml was found too toxic for embryo survival, so exposure studies were conducted at 1 ug/ml at both pre-gastrulation and post-gastrulation times. Embryos exposed at pre-gastrulation had lower PGC abundance whereas embryos exposed at post-gastrulation remained the same.
MXC proved less lethal, with embryos surviving up to 100 ug/ml. Exposure at neither pre- nor post-gastrulation affected PGC abundance. However, melanocytes disappeared and earlier locomotion was observed (data not shown). Histology analysis of MXC-exposed embryos showed no significant difference in musculature when comparing the head, trunk, and gut.



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